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Initial Genome Scan of the NIMH Genetics Initiative Bipolar Pedigrees: Chromosomes 4, 7, 9, 18, 19, 20, and 21q

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An initial genome scan was performed on 540 individuals from 97 families segregating bipolar disorder, collected through the National Institutes of Mental Health Genetics Initiative. We report here affected-sib-pair (ASP) data on 126 marker loci (≈68,000 genotypes) mapping to chromosomes 4, 7, 9, 18, 19, 20, and 21q, under three affection status models. Modest increases in identical-by-descent (IBD) allele sharing were found at the following loci: D4S2397 and D4S391 ($P < 0.05$) on 4p, D4S1647 ($P < 0.05$) on 4q, D7S1802 and D7S1869 (low $P = 0.01$) on 7p, D9S302 ($P = 0.004$) on 9q, and D20S604 on 20p and D20S173 on 20q ($P \leq 0.05$). In addition, five markers on 7q displayed increased IBD sharing ($P = 0.046$–0.002). Additional ASP analyses on chromosomes 18 and 21q marker data were performed using disease phenotype models defined previously. On chromosome 18, only D18S40 on 18p and D18S70 on 18q yielded a slight elevation in allele sharing ($P = 0.02$), implying that the reported linkages in these regions were not confirmed. On chromosome 21q, a cluster of markers within an ≈9 cM interval: D21S1254, D21S65, D21S1440, and D21S1255 exhibited excess allele sharing ($P = 0.041$–0.008). Multilocus data on overlapping marker quartets, from D21S1265 to D21S1255, which were consistent with increased IBD sharing ($P < 0.01$, with a low of 0.0009), overlapped a broad interval of excess allele sharing reported previously, increasing support for a susceptibility locus for bipolar disorder on 21q. Am. J. Med. Genet. 74:254–262, 1997.

KEY WORDS: bipolar pedigree; genome scan; bipolar disorder; linkage

INTRODUCTION

A number of genomic regions have been proposed to be either linked or implicated in bipolar disorder [Risch and Botstein 1996; Nurnberger et al., 1997]. Although there have been recent replications of linkage findings, nonreplications at the same loci have also been reported, possibly due to random statistical fluctuations associated with sample sizes that have insufficient power to detect linkage to genes with small effects [Suarez et al., 1994; Hauser et al., 1996]. In an attempt to identify additional susceptibility regions for bipolar disorder as well as to test prior hypotheses a genome-wide screening was conducted on a new independent pedigree sample. This series consists of 97 families

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with 540 individuals, collected by four collaborating centers of the National Institute of Mental Health (NIMH) Genetics Initiative, which included the Clinical Neurogenetics Branch (CNG)/NIMH, Indiana University, Johns Hopkins University, and Washington University. In the present report, we confine our comments to chromosomes 4, 7, 9, 18, 19, 20, and 21q, all of which we are screening in our site, the CNG. The other chromosomes are being done by our collaborators [Edenberg et al.,1997; Rice et al.,1997; Stine et al., 1997].

Evidence for linkage to the pericentromeric region of chromosome 18 has been reported in the 22 CNG pedigrees [Berrettini et al., 1994]. This initial finding was supported by that of Stine et al.[1995], who additionally proposed another possible locus on the proximal region of 18q. Recently, Freimer et al. [1996] reported evidence for a susceptibility gene close to the tip of 18q. In effect, these three studies implicated virtually the entire chromosome 18.

A vulnerability locus on 21q22.3 was previously proposed by Straub et al. [1994]. Gurling et al. [1995] presented data consistent with this proposal. Recently, we extended these studies on the 22 CNG series through the use of the affected-sib-pair (ASP) method of analysis, and this provided confirmation of linkage on 21q [Detera-Wadleigh et al., 1996], based on Lander and Kruglyak's [1995] criteria. This study also demonstrated for the first time that excess IBD allele-sharing extended from the distal to the proximal regions of chromosome 21q [Detera-Wadleigh et al., 1996].

Recently, Blackwood et al. [1994] reported linkage of markers on 4p in a single family from Scotland. So far, no replication of this finding has been reported. By parametric linkage analysis, we previously found a restriction fragment length polymorphism (RFLP) marker on 7q that yielded a lod score >3, but the use of flanking microsatellite markers produced negative lod scores, thus failing to confirm this initial result [Detera-Wadleigh et al., 1994].

The availability of the present pedigree series permitted further examination of the reported linkages on chromosomes 4p, 18, and 21q in an independent sample. In this study, we report excess allele sharing on chromosome 21q that is consistent with the possible existence of a susceptibility locus in this region, lack of confirmation of reported linkages on chromosomes 4 and 18, and newly detected chromosomal regions with increased IBD sharing.

MATERIALS AND METHODS

Genotyping

A total of 540 individuals from 97 families were genotyped with each marker. Because of Mendelian inconsistencies, one family was not included in the linkage calculations. Marker allele frequencies were estimated from data derived from this series.

The majority of the short tandem repeat markers used in this study detected tetranucleotide polymorphisms. These, along with dinucleotide and trinucleotide repeat markers, were included in the Cooperative Human Linkage Center (CHLC) Human Screening Set/Weber version 6 (available from Research Genetics). Other markers were taken from the published maps of Genethon [Gyapay, 1994] and CHLC [1994, 1995], recent updates of which are available via the Internet, and radiation hybrid maps from the Stanford Human Genome Center (SHGC) [Cox et al., 1990]. Both unlabelled and fluorescently labelled primers were purchased from Research Genetics, Inc.

Both manual and automated genotyping procedures were used. For radioactive genotyping, only one amplification condition was employed for all markers, as described earlier [Detera-Wadleigh et al., 1996]. Both the Perkin-Elmer Cetus GeneAmp 9600 and Bio-Oven Biotherm III thermocyclers were used. Size markers and controls consisted of genomic DNA from two CEPH individuals, a 123-bp ladder, and sequencing ladders. Autoradiograms were scored by two independent readers, both of whom were blind to diagnosis.

The Perkin-Elmer GeneAmp System 9600 thermocycler was used exclusively for automated genotyping. Polymerase chain reaction (PCR) conditions established by ABI-Perkin-Elmer Cetus were utilized in amplifications involving fluorescent primers. PCR was conducted separately for each marker. Multiplexing, which was accomplished during electrophoresis, was limited to 11 tetranucleotide repeat markers per lane since a higher number resulted in overlaps of peaks from different markers.

ABI-generated data was analyzed by use of the Genescan program which assigned sizes in bp to the respective peaks based on internal lane standards. Genescan data were imported into the Genotyper program to review peaks and assign alleles. The printout of peaks for each marker was examined individually to ensure that the size for all peaks were indicated.

Primers for D18S64 were redesigned to delete complementary ends. This resulted in sharper bands with fewer stutters, thus enabling definitive band designations. Oligomers were synthesized by Bioserve Biotechnologies, Inc.

Genotyping for D18S32 was performed using a minisatellite probe cMS615 [Armour et al., 1990]. The probe was labelled by the random primer method using α^{32}PdCTP to a specific activity of 10^9 cpm per μg. In this experiment genomic DNA was extracted from whole blood by the nonorganic, nonenzymatic "rapid method" [Lahiri and Nurnberger, 1991]. After hybridization of Southern blots generated through Hinf-I digestions, six different fragment sizes (range: 4.5–3.35 kb) were evident (frequencies: 0.43, 0.07, 0.06, 0.40, 0.03, and 0.02, respectively).

Affection Status Models

Diagnostic categories (RDC and DSM III-R) were agreed upon by collaboration (CNG/NIMH, Indiana University, Johns Hopkins University, and Washington University) in the NIMH Genetics Initiative; three models were defined for the affected phenotype (NIMH Genetics Initiative Bipolar Group, 1997]: collaborative models I, II, and III. The approximate numbers of independent ASPs under Models I, II and III were 90, 130, and 175, respectively. These phenotypic categories were used in the analysis of all marker genotype data.

In our attempt to find possible support for our previous findings on chromosomes 18 [Berrettini et al., 1994] and 21q [Detera-Wadleigh et al., 1996] in a separate pedigree sample, analysis was done also by using previously described diagnostic definitions, i.e., modified RDC [Berrettini et al. 1991], and affection status models employed in these earlier studies. These models comprised affection status model (ASM)-I, which included bipolar I (BPI), bipolar II (BPII) with major depression, and schizoaffective illness; and ASM-II, which included ASM I plus recurrent unipolar depression. The approximate numbers of independent ASPs under ASM-I and -II models were 120 and 150, respectively.

Linkage Analysis

ASP analysis [Blackwelder and Elston, 1985] was performed by use of the SIBPAL program, 1996 version 2.7.3, of the S.A.G.E. Release 2.2 package [S.A.G.E., 1994]. Mean IBD allele-sharing and corresponding *P*-values were computed. IBD allele-sharing for the full sibs only are reported in this study; half-sib data are not presented. *P*-values were corrected for the number of independent sib-pairs. Multilocus ASP analysis was performed using the MIM program [Goldgar, 1990; Goldgar and Oniki, 1992; Goldgar et al., 1993].

To evaluate the significance levels of the ASP data, we employed the guidelines proposed by Lander and Kruglyak [1995] to assign suggestive linkage, significant linkage, and confirmation of prior evidence of linkage.

RESULTS

Chromosome 4

A total of 26 markers were used to screen chromosome 4 (Table I). Intermarker spacing was relatively dense, with the exception of a large gap of ≈31 cM, between D4S2623 and D4S1644 on 4q. Three markers on 4p (D4S394, D4S1582, and D4S403), which yielded lod scores >3 in a large Scottish family with bipolar disorder [Blackwood et al., 1996], were included in this analysis. However, these and other markers spanning the entire chromosome 4 did not reveal any highly significant increase in allele-sharing (Table I). At D4S2397, which is ≈20 cM proximal to D4S403, we found a modest increase in IBD sharing, with *P*-values of 0.023 and 0.027 under collaborative models II and III, respectively. Similarly, D4S391, which is ≈3 cM distal to D4S2397, yielded a *P*-value of 0.045, under model III. On 4q, only D4S1647 yielded slightly increased allele-sharing under model I ($P = 0.036$).

Chromosome 7

We typed 26 markers to scan chromosome 7 (Table I). Gaps of ≈15 cM were located in the distal portion of 7q. On 7p, ASP analysis yielded $P < 0.05$ for both D7S1802 and D7S1869 under collaborative models II and III, but data from a marker mapping between these two loci did not support increases in allele-sharing. Markers on chromosome 7q: D7S524, D7S1799, D7S501, D7S490, and D7S2195 displayed *P*-values ranging from 0.046 to 0.002 under phenotype Model III. The first four markers cluster within ≈25 cM region of 7q.

Chromosomes 9, 19, and 20

On chromosomes 9, 19, and 20, we analyzed 13, 11, and 8 marker loci, respectively (Table II). Intermarker gaps ≥15 cM were between D9S925 and D9S741, D9S922 and D9S910, D19S1034 and D19S586, D20S95 and D20S604, and D20S481 and D20S173. On chromosome 9q, D9S302 yielded *P*-values of 0.004 and 0.025 under collaborative models II and III, respectively (Table II), and flanking markers are currently being examined. A weak increase in allele-sharing ($P \leq 0.05$) under model I was displayed by isolated markers: D20S604 on 20p and D20S173 on 20q (Table I). So far, no signals were evident on chromosome 19.

Chromosome 18

The present study permitted further examination of previous reports of linkage on chromosome 18 in an independent sib-pair family collection. Some of the markers used here were the same as those employed in earlier studies [Berrettini et al., 1994; Stine et al., 1995; Freimer et al., 1996].

We typed a total of 23 markers spanning the entire length of chromosome 18 (Tables III, IV). We found that two of these markers (D18S53 and D18S542) detected the identical polymorphism [Esterling et al., unpublished data]; here, we report only the data for D18S542 and, therefore, 22 markers on chromosome 18. Under the collaborative models, only D18S70 exhibited a modest increase in allele-sharing ($P = 0.024$) under the most stringent phenotype definition (Table III). In an attempt to replicate the previous findings of Berrettini et al. [1994], we used the CNG affection status models ASM-I and -II. We found that D18S40 showed a slightly increased proportion of shared alleles in affected sibs ($P = 0.022$, for ASM II) (Table IV). A similar pattern was exhibited by D18S70 ($P = 0.044$, for ASM-I).

Chromosome 21q

Excess allele sharing encompassing the proximal and distal regions of 21q was found in our original 22-CNG pedigree series [Detera-Wadleigh et al., 1996]. This prior study also replicated linkage reported by Straub et al. [1994]. We used 20 markers mapping to the long arm of chromosome 21, 13 of which were identical to those employed in our previous study [Detera-Wadleigh et al., 1996] (Tables 3 and 4). The seven new markers were D21S1442, D21S269, D21S260, APP, D21S1265, D21S1254, and D21S1255. ASP analysis showed that under collaborative model I, D21S1255 displayed a slight increase in allele-sharing ($P < 0.05$) (Table III). A similar pattern was shown by D21S1254 and D21S65 under model II, and by D21S65 and D21S1252 under model III.

ASP data under the CNG affection status models, ASM-I and -II, revealed excess allele-sharing in four markers clustered within a region encompassing ≈8 cM: D21S1254, D21S65, D21S1440, and D21S1255, with nominal *P*-values under ASM-I of 0.019, 0.020,

TABLE I. ASP Data on Chromosomes 4 and 7 Under Three Collaborative Affected Phenotype Models*

Locus		Model I			Model II			Model III		
Chromosome 4	cM	No. ASP	IBD	*P*	No. ASP	IBD	*P*	No. ASP	IBD	*P*
D4S2366	0	116	0.494	1.000	188	0.499	1.000	262	0.482	1.000
D4S394	4	110	0.475	1.000	186	0.487	1.000	264	0.483	1.000
D4S1582	11	114	0.482	1.000	188	0.489	1.000	265	0.481	1.000
D4S403	14	118	0.512	0.326	191	0.501	0.481	270	0.491	1.000
D4S1601	17	106	0.478	1.000	173	0.488	1.000	228	0.487	1.000
D4S2639	24	116	0.527	0.183	192	0.517	0.228	271	0.506	0.380
D4S1551	26	109	0.487	1.000	180	0.500	1.000	254	0.506	0.348
D4S391	31	110	0.532	0.142	179	0.527	0.126	254	0.534	**0.045**[a]
D4S2397	34	111	0.525	0.172	183	0.541	0.023	261	0.534	**0.027**[a]
D4S2408	39	116	0.523	0.205	189	0.533	0.052	267	0.523	0.091
D4S2369	52	108	0.519	0.246	180	0.529	0.094	253	0.531	0.052
D4S1627	55	114	0.492	1.000	187	0.514	0.281	261	0.524	0.086
GATA28F03	64	115	0.522	0.184	187	0.519	0.165	264	0.519	0.107
D4S2367	72	119	0.516	0.281	186	0.524	0.153	263	0.514	0.264
D4S3243	84	120	0.520	0.197	193	0.510	0.300	269	0.499	1.000
D4S2361	88	114	0.527	0.149	184	0.519	0.181	259	0.502	1.000
D4S1647	100	118	0.546	**0.036**[a]	185	0.516	0.228	263	0.508	0.374
D4S2623	**110**[b]	118	0.513	0.330	190	0.512	0.306	267	0.500	0.491
D4S1644	**141**[b]	111	0.506	0.414	183	0.509	0.337	262	0.512	0.242
D4S1625	145	106	0.534	0.132	174	0.500	0.499	248	0.503	0.405
D4S1629	159	118	0.475	1.000	194	0.480	1.000	273	0.475	1.000
D4S2368	167	115	0.456	1.000	189	0.484	1.000	266	0.488	1.000
D4S2431	175	109	0.497	1.000	179	0.496	1.000	252	0.496	1.000
D4S2417	183	121	0.506	0.410	197	0.495	1.000	277	0.496	1.000
D4S408	197	117	0.500	1.000	193	0.509	0.339	273	0.502	0.428
D4S1652	209	117	0.478	1.000	193	0.500	0.498	269	0.503	0.440
Chromosome 7										
D7S2201	0	118	0.491	1.000	192	0.473	1.000	270	0.479	1.000
D7S664	6	107	0.500	0.323	176	0.500	0.407	245	0.510	0.367
D7S507	14	110	0.516	0.292	182	0.515	0.248	261	0.510	0.292
D7S1795	21	111	0.512	0.297	183	0.509	0.323	258	0.496	1.000
D7S1802	25	119	0.540	0.066	194	0.550	**0.013**[a]	267	0.534	**0.027**[a]
D7S1808	34	112	0.540	0.095	186	0.506	0.304	263	0.509	0.351
D7S1869	39	109	0.550	**0.023**[a]	179	0.529	**0.034**[a]	244	0.535	**0.013**[a]
D7S817	44	118	0.474	1.000	194	0.481	1.000	265	0.489	1.000
GATA31A10	52	116	0.471	1.000	190	0.481	1.000	267	0.492	1.000
D7S1818	68	119	0.493	1.000	195	0.496	1.000	273	0.495	1.000
D7S1830	71	113	0.488	1.000	189	0.498	1.000	269	0.501	0.483
D7S2204	87	114	0.484	1.000	180	0.508	0.279	257	0.513	0.238
D7S2212	94	116	0.505	0.426	187	0.510	0.297	262	0.516	0.172
D7S820	96	115	0.504	0.451	187	0.511	0.321	265	0.527	0.092
D7S524	100	106	0.512	0.225	178	0.530	0.112	249	0.543	**0.013**[a]
D7S821	109.5	116	0.529	0.167	189	0.534	0.075	269	0.530	0.059
D7S1799	113.5	115	0.530	0.166	187	0.530	**0.049**[a]	263	0.551	**0.002**[a]
D7S501	115.5	110	0.526	0.161	182	0.523	0.143	254	0.535	**0.029**[a]
D7S471	120.5	99	0.496	1.000	168	0.491	1.000	240	0.518	0.193
D7S490	125.5	111	0.495	1.000	178	0.498	1.000	248	0.531	**0.046**[a]
GATA44F09	126	108	0.481	1.000	179	0.482	1.000	251	0.507	0.280
D7S1804	**138**[b]	119	0.504	0.338	193	0.506	0.316	272	0.520	0.146
D7S1824	**153**[b]	118	0.529	0.177	190	0.520	0.216	267	0.526	0.090
D7S2195	**158**[b]	107	0.567	**0.016**[a]	174	0.525	0.167	251	0.538	**0.026**[a]
D7S1805	**173**[b]	111	0.526	0.193	181	0.509	0.339	249	0.522	0.121
D7S1826	174	118	0.479	1.000	192	0.490	1.000	271	0.501	0.412

*Single locus ASP analysis of marker data. All *P* values are corrected for the number of independent sib-pairs. Intermarker distances and order were deduced from publicly (Internet) available maps and those published by Genethon [Gyapay et al., 1994], CHLC and Weber/CHLC screening set (version 6), and the SHGC database.

[a]$P \leq 0.05$.

[b]Sites with gaps $\geq$ 15 cM.

0.016, and 0.033, respectively, and 0.080, 0.008, 0.033, and 0.033 under ASM-II, respectively (Table IV).

To examine the intermarker interval within the region of increased allele sharing, we performed multilocus ASP analysis on overlapping marker quartets using both CNG and collaborative affected phenotype definitions. All locus quartets spanning the interval from the proximal locus, D21S1265, to the more distal locus, D21S1255, showed excess IBD sharing ($P < 0.01$) under ASM-I and -II (Table V). The lowest *P*-value (0.0009) was shown by the quartet D21S1254-S65-S1252-S1440 under ASM-II. Almost identical multilocus data were found for all collaborative models (Table V).

TABLE II. ASP Data on Chromosomes 9, 19, and 20 Under Three Collaborative Affected Phenotype Models*

Locus		Model I			Model II			Model III		
Chromosome 9	cM	No. ASP	IBD	*P*	No. ASP	IBD	*P*	No. ASP	IBD	*P*
GATA62F03	0	110	0.497	1.000	185	0.482	1.000	261	0.482	1.000
D9S925	**18**[b]	112	0.530	0.147	187	0.516	0.242	266	0.501	0.478
D9S741	**35**[b]	106	0.460	1.000	162	0.460	1.000	234	0.470	1.000
D9S1118	48	116	0.504	0.439	192	0.502	0.456	271	0.500	0.459
D9S301	58	117	0.487	1.000	193	0.481	1.000	272	0.492	1.000
D9S1122	65	110	0.520	0.174	179	0.524	0.116	251	0.520	0.100
D9S922	**70**[b]	111	0.488	1.000	183	0.517	0.209	260	0.514	0.218
D9S910	**95**[b]	118	0.520	0.211	193	0.509	0.330	272	0.500	0.403
D9S930	112	114	0.498	1.000	190	0.531	0.119	270	0.515	0.171
D9S302	115	112	0.541	0.120	187	0.568	**0.004**[a]	264	0.541	**0.025**[a]
D9S934	120	106	0.510	0.316	178	0.527	0.070	256	0.524	0.077
D9S290	132	98	0.540	0.084	154	0.530	0.150	214	0.520	0.198
D9S915	139	106	0.530	0.114	138	0.520	0.182	256	0.510	0.338
Chromosome 19										
D19S247	1	103	0.518	0.274	177	0.505	0.407	251	0.502	0.408
D19S1034	**13**[b]	109	0.489	1.000	178	0.498	1.000	246	0.489	1.000
D19S586	**28**[b]	69	0.450	1.000	105	0.450	1.000	140	0.460	1.000
D19S714	36	118	0.496	1.000	188	0.488	1.000	264	0.510	0.329
D19S433	45	105	0.485	1.000	175	0.470	1.000	244	0.498	1.000
D19S587	51	110	0.475	1.000	167	0.465	1.000	241	0.479	1.000
D19S178	61	108	0.500	1.000	180	0.500	0.481	255	0.530	0.092
D19S246	71	117	0.520	0.220	190	0.534	0.126	268	0.518	0.142
D19S601	78	103	0.484	1.000	176	0.490	1.000	252	0.502	0.465
D19S589	83	107	0.510	0.341	179	0.500	0.432	251	0.496	1.000
D19S254	95	116	0.467	1.000	187	0.464	1.000	262	0.464	1.000
Chromosome 20										
D20S473	0	116	0.530	0.053	187	0.523	0.071	265	0.500	0.386
D20S95	**9**[b]	103	0.530	0.205	173	0.520	0.159	245	0.530	0.095
D20S604	**27**[b]	100	0.550	**0.050**[a]	164	0.525	0.068	237	0.521	0.125
D20S470	33	106	0.532	0.162	173	0.510	0.345	244	0.526	0.141
D20S477	41	102	0.490	1.000	167	0.480	1.000	239	0.500	1.000
D20S478	49	100	0.460	1.000	166	0.460	1.000	239	0.480	1.000
D20S481	**58**[b]	112	0.470	1.000	183	0.491	1.000	256	0.511	0.257
D20S173	**91**[b]	102	0.560	**0.019**[a]	167	0.520	0.169	235	0.520	0.160

*Single locus ASP analysis of marker data. All *P* values are corrected for the number of independent sib-pairs. Intermarker distances and order were deduced from publicly (Internet) available maps and those published by Genethon [Gyapay et al., 1994], CHLC and Weber/CHLC screening set (version 6), and the SHGC database.
[a]$P \leq 0.05$.
[b]Sites with gaps $\geq$ 15 cM.

DISCUSSION

We analyzed a total of 126 marker loci (≈68,000 genotypes) mapping to chromosomes 4, 7, 9, 18, 19, 20, and 21q in order to screen for evidence of new susceptibility regions as well as to find support of previously hypothesized loci for bipolar disorder. ASP analysis revealed weak signals ($P > 0.01$) in two adjacent markers on 4p and one marker on 4q; therefore, in this pedigree sample, the proposed linkage on chromosome 4p [Blackwood et al. 1996] is not confirmed based on Lander and Kruglyak's criteria [1995]. Similarly, positive results in two different studies that implicated intervals on both 18p and 18q [Berrettini et al. 1994; Stine et al. 1995] have not been supported in these families. The increase in allele-sharing at D18S40 on 18p failed to meet the level of significance for replication [Lander and Kruglyak, 1995]. Since earlier studies have shown a parental origin effect in bipolar disorder [McMahon et al., 1995] and since a paternal effect has been noted on this chromosome in two separate studies [Stine et al. 1995; Gershon et al. 1996], we grouped the families into maternal and mixed maternal/paternal subtypes based on the history of transmission of illness. ASP analysis performed separately on these two groups failed to reveal significance, consistent with the absence of replication. From our studies on the 22 CNG pedigrees, we found that the highest locus-specific recurrence risk [Risch, 1987] in the pericentromeric region of chromosome 18 is relatively low (lambda = 1.6) [Detera-Wadleigh et al., 1996], implying a weak effect susceptibility gene. Given the predicted relatively small effect of this locus to overall susceptibility, the lack of power to replicate could be due to insufficient sample size [Suarez et al., 1995; Hauser et al. 1996].

Freimer et al. [1996] reported linkage and association of bipolar disorder with markers on 18q22-q23 in Costa Rican pedigrees. In the present family sample, one of these marker loci, D18S70, located close to the telomere of 18q, displays a *P*-value of 0.024 using the most stringent phenotype definition, which included BPI and SA. This level of significance does not meet the criteria for confirmation of linkage as previously pro-

TABLE III. ASP Data on Chromosomes 18 and 21q Under Three Collaborative Affected Phenotype Models*

Locus		Model I			Model II			Model III		
Chromosome 18	cM	No. ASP	IBD	*P*	No. ASP	IBD	*P*	No. ASP	IBD	*P*
D18S59	0	107	0.470	1.000	173	0.498	1.000	237	0.488	1.000
D18S481	7	92	0.505	0.486	155	0.499	1.000	225	0.490	1.000
D18S976	**18**[b]	118	0.498	1.000	190	0.497	1.000	263	0.497	1.000
D18S843	**34**[b]	117	0.500	1.000	192	0.513	0.281	270	0.524	0.085
D18S1150	45	110	0.530	0.156	179	0.520	0.161	254	0.510	0.270
D18S542	46	115	0.490	1.000	190	0.493	1.000	261	0.496	1.000
D18S1116	46.5	117	0.500	1.000	189	0.517	0.265	264	0.522	0.117
GNAL (PCR5)	47	118	0.470	1.000	191	0.490	1.000	270	0.500	1.000
D18S37	48.3	116	0.532	0.194	185	0.523	0.187	261	0.515	0.177
D18S40	48.9	117	0.544	0.115	189	0.525	0.206	269	0.528	0.074
D18S852	49.2	118	0.499	1.000	194	0.510	0.366	272	0.511	0.185
D18S877	63.2	116	0.507	0.399	191	0.500	0.492	270	0.503	0.435
D18S535	74.2	118	0.519	0.345	192	0.514	0.331	269	0.504	0.403
D18S851	84.2	114	0.467	1.000	188	0.475	1.000	265	0.471	1.000
D18S858	90.2	114	0.496	1.000	189	0.502	1.000	265	0.487	1.000
D18S64	**94.2**[b]	107	0.499	1.000	181	0.493	1.000	258	0.485	1.000
D18S541	**124.2**[b]	107	0.481	1.000	171	0.478	1.000	239	0.472	1.000
D18S469	124.7	104	0.466	1.000	175	0.493	1.000	239	0.488	1.000
D18S554	131.7	101	0.470	1.000	163	0.480	1.000	227	0.495	1.000
D18S70	139.7	101	0.560	**0.024**[a]	165	0.540	0.069	231	0.520	0.114
D18S844	140.2	113	0.476	1.000	186	0.485	1.000	262	0.483	1.000
D18S32	N/A	68	0.500	1.000	101	0.530	0.182	136	0.530	0.111
Chromosome 21q										
D21S1436	0	106	0.497	0.497	173	0.489	1.000	247	0.485	1.000
D21S1437	4	108	0.467	1.000	174	0.469	1.000	250	0.481	1.000
GRIK (OLI-1/3F)	8	115	0.493	1.000	188	0.515	0.217	265	0.507	0.315
APP	8.5	106	0.505	0.424	167	0.513	0.269	234	0.496	1.000
D21S269	9.5	115	0.505	0.419	184	0.501	0.483	259	0.493	1.000
D21S1435	9.9	109	0.521	0.231	182	0.503	0.440	248	0.496	1.000
D21S260	10.0	107	0.530	0.120	176	0.508	0.340	249	0.507	0.337
D21S1442	10.6	100	0.500	0.377	160	0.510	0.275	227	0.490	1.000
D21S1265	13.6	112	0.508	0.390	183	0.517	0.224	262	0.505	0.388
D21S1270	14.4	117	0.495	1.000	182	0.498	1.000	257	0.487	1.000
D21S1254	18.8	110	0.533	0.094	179	0.534	**0.031**[a]	250	0.516	0.145
D21S65	20.8	109	0.536	0.112	177	0.540	**0.046**[a]	256	0.540	**0.032**[a]
D21S1252	23.0	118	0.535	0.088	189	0.524	0.119	262	0.528	**0.050**[a]
D21S1440	23.3	114	0.534	0.088	182	0.524	0.129	258	0.521	0.110
D21S1255	28.0	108	0.550	**0.038**[a]	181	0.524	0.123	253	0.521	0.111
HMG14	31.0	105	0.512	0.344	177	0.507	0.379	255	0.504	0.407
D21S266	37.0	116	0.495	1.000	189	0.483	1.000	267	0.494	1.000
D21S212	40.0	115	0.503	0.457	189	0.478	1.000	261	0.491	1.000
D21S171	44.8	113	0.507	0.392	189	0.472	1.000	265	0.483	1.000
PFKL (PCR1)	45.8	115	0.488	1.000	188	0.471	1.000	260	0.475	1.000

*Single locus ASP analysis of marker data. All *P* values are corrected for the number of independent sib-pairs. Intermarker distances and order were deduced from publicly (Internet) available maps and those published by Genethon [Gyapay et al., 1994], CHLC and Weber/CHLC screening set (version 6), and the SHGC database. Primers for indicated loci are in parentheses.

[a]$P \leq 0.05$.

[b]Sites with gaps $\geq$ 15 cM.

posed [Lander and Kruglyak, 1995]. In the study by Freimer et al. [1996], however, only BPI was included in the affected category; thus, analysis of our data using this affection status model would be important.

Our previous study of chromosome 21q on a separate set of pedigrees [Detera-Wadleigh et al., 1996] supported prior evidence for linkage on 21q22.3 [Straub et al., 1994]. Our study further revealed that the region of excess allele sharing was broad, extending from the proximal to the distal portion of the long arm of the chromosome. The present analysis of 97 bipolar families depicts a cluster of markers on 21q that displays increased allele-sharing in ASPs, overlapping the region of excess allele-sharing shown previously [Detera-Wadleigh et al., 1996]. These findings reinforce the idea that the region of significance on chromosome 21q is large [Detera-Wadleigh et al., 1996]. Within this broad interval, D21S65 yields a single locus ASP *P*-value of 0.008. Multilocus ASP analyses on overlapping marker quartets indicate significant allele-sharing (low *P* of 0.0009) and is, therefore, consistent with the evidence of a susceptibility locus for bipolar disorder on 21q.

In this study on a new independent pedigree collection, we also detected several new signals, exhibiting increased allele-sharing, such as those on chromosomes 7, 9, and 20. Although the level of significance in these regions does not represent either suggestive or

TABLE IV. ASP Data on Chromosomes 18 and 21q Under Two CNG Affected Phenotype Models*

Locus	ASM-I			ASM-II		
Chromosome 18	No. ASP	IBD	*P*	No. ASP	IBD	*P*
D18S59	159	0.490	1.000	190	0.496	1.000
D18S481	141	0.500	1.000	176	0.489	1.000
D18S976	178	0.505	0.416	218	0.502	0.458
D18S843	177	0.521	0.177	218	0.530	0.097
D18D1150	168	0.520	0.141	211	0.520	0.136
D18S542	175	0.503	0.428	209	0.511	0.295
D18S1116	174	0.500	0.432	212	0.520	0.175
GNAL	177	0.500	1.000	220	0.510	0.355
D18S37	170	0.520	0.101	210	0.522	0.099
D18S40	177	0.530	0.134	220	0.540	**0.022**[a]
D18S852	179	0.507	0.460	220	0.509	0.260
D18S877	176	0.494	1.000	218	0.506	0.379
D18S535	177	0.510	0.319	219	0.510	0.292
D18S851	175	0.480	1.000	216	0.480	1.000
D18S858	174	0.510	0.339	215	0.498	1.000
D18S64	168	0.490	1.000	210	0.497	1.000
D18S541	163	0.490	1.000	204	0.480	1.000
D18S469	158	0.500	1.000	195	0.500	1.000
D18S554	153	0.470	1.000	187	0.480	1.000
D18S70	149	0.540	**0.044**[a]	187	0.530	0.101
D18S844	172	0.491	1.000	214	0.491	1.000
D18S32	92	0.530	0.173	108	0.540	0.089
Chromosome 21q						
D21S1436	160	0.500	1.000	199	0.490	1.000
D21S1437	158	0.473	1.000	199	0.484	1.000
GRIK	173	0.512	0.270	214	0.510	0.385
APP	152	0.515	0.246	185	0.508	0.345
D21S269	171	0.500	0.449	212	0.500	0.403
S21S1435	165	0.504	0.428	206	0.502	0.463
D21S260	163	0.520	0.160	204	0.520	0.137
D21S1442	150	0.510	0.302	182	0.500	0.455
D21S1265	167	0.516	0.244	210	0.515	0.234
D21S1270	170	0.498	1.000	211	0.493	1.000
D21S1254	166	0.540	**0.019**[a]	208	0.530	**0.041**[a]
D21S65	164	0.549	**0.020**[a]	206	0.547	**0.008**[a]
D21S1252	175	0.529	0.097	215	0.532	**0.048**[a]
D21S1440	167	0.550	**0.018**[a]	209	0.540	**0.033**[a]
D21S1255	167	0.540	**0.033**[a]	206	0.540	**0.033**[a]
HMG14	162	0.522	0.188	205	0.510	0.331
D21S266	174	0.491	1.000	216	0.495	1.000
D21S212	174	0.490	1.000	216	0.493	1.000
D21S171	174	0.484	1.000	216	0.486	1.000
PFKL	174	0.480	1.000	214	0.482	1.000

*Single locus ASP analysis of marker data. All *P* values are corrected for the number of independent sib-pairs. Intermarker distances and order were deduced from publicly (Internet) available maps and those published by Genethon [Gyapay et al., 1994], CHLC and Weber/CHLC screening set (version 6), and the SHGC database.
[a]$P \leq 0.05$.

definitive linkage [Lander and Kruglyak, 1995], they serve as sites for further investigation, such as typing of flanking markers and multilocus analysis, in this and other independent series.

We are continuing the chromosomal scans in order to generate a dense screening of this sib-pair collection. Risch and Botstein [1996] noted that while a major locus for bipolar disorder may exist, it remains elusive if it does. Therefore, it is generally believed that bipolar disorder is caused by multiple genes with modest contributions to the overall susceptibility. The possibility exists that we have missed the detection of significant linkage, particularly to susceptibility loci with minor effects. Hauser et al. [1996] recently performed simulations to evaluate power of linkage detection for a given number of sib-pairs by use of a model-free lod score method, parameterized by relative recurrence risk. For 100 sib-pair families with one or both parents genotyped (which approximates the NIMH Genetics Initiative pedigree collection), and an intermarker distance of 10 cM (with a disease locus in the middle of the interval), they found expected maximum lod scores of 1.45, 1.91, and 3.92 under recurrence risk ratios of 1.6, 2.0, and 3.0, respectively. Power rose dramatically when the number of ASPs was augmented severalfold, the relative recurrence risk increased and intermarker distance decreased. These data predict a relatively low power of the current series to identify significant evi-

TABLE V. Chromosome 21q: Multilocus ASP Data

	ASM-I		ASM-II		Model I		Model II		Model III	
Locus quartet	IBD	*P*	IBD	*P*	IBD	*P*	IBD	*P*	IBD	*P*
D21S1265 D21S1270 D21S1254 D21S65	0.54	**0.0056**	0.54	**0.0036**	0.54	**0.027**	0.54	**0.0075**	0.53	**0.018**
D21S1270 D21S1254 D21S65 D21S1252	0.55	**0.0047**	0.55	**0.0015**	0.56	**0.0053**	0.54	**0.0055**	0.54	**0.0065**
D21S1254 D21S265 D21S1252 D21S1440	0.55	**0.0025**	0.55	**0.0009**	0.56	**0.0045**	0.54	**0.0073**	0.54	**0.0032**
D21S65 D21S1252 D21S1440 D21S1255	0.55	**0.0075**	0.55	**0.0026**	0.55	**0.023**	0.53	**0.031**	0.54	**0.0087**

dence for weak-effect susceptibility loci. Thus, weak signals found in the present sample may turn out to be important when a much larger series is examined.

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